A Case-Control Study of Risk Factors for Invasive Cervical Cancer among U.S. Women Exposed to Oncogenic Types of Human Papillomavirus

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Abstract

Oncogenic human papillomavirus (HPV) infections, the necessary cause of most cervical cancers, are common and usually clear within 1 to 2 years. Identifying cofactors that lead to cancer among HPV-infected women has depended mainly on case-control studies defining HPV by DNA testing. DNA testing assesses only current infection; thus, concerns about residual confounding remain. To assess cofactors, we used seropositivity to five oncogenic HPV types as a marker of past exposure and confined our analysis to seropositive controls compared with cancer cases. Study subjects had participated in a multicenter U.S. case-control study conducted in the early 1980s. The detailed questionnaire and stored sera for 235 cases of squamous carcinoma and 486 controls motivated the reanalysis. We measured antibodies to HPV types 16, 18, 31, 45, and 52. Independent, significant predictors of seropositivity among controls included numbers of sexual partners, Black race, and oral contraceptive use. Condom use was protective. Among HPV-exposed women, Papanicolaou screening, Black race, and yeast infection were significantly associated with reduced cancer risk. Current smoking was associated with a 2-fold increase in risk; there were independent, significant trends of increased risk with numbers of cigarettes smoked (*P* for trend = 0.003) and years of smoking (*P* for trend = 0.01). Other significant predictors of increased risk included low education and income and history of nonspecific genital infection. Unlike recent HPV DNA-based investigations, based on the use of HPV-seropositive controls in this study, oral contraceptive use was unrelated to the risk of cervical cancer and multiparity was only weakly related to risk. It is particularly worth considering further why studies of different designs are inconsistent regarding the effect of oral contraceptive use. (Cancer Epidemiol Biomarkers Prev 2004;13(10):1574–82)

Introduction

Infection with 1 of ~15 oncogenic types of human papillomavirus (HPV) is the necessary cause of virtually all cases of cervical cancer worldwide (1). HPV types 16, 18, 31, and 45 account for three quarters of cases. However, most infections with oncogenic HPVs are benign and become undetectable by even sensitive DNA detection methods within 2 years (2). A current epidemiologic challenge is to identify etiologic cofactors that lead to HPV persistence and neoplastic progression and to clarify how they contribute to carcinogenesis (3, 4).

Epidemiologists studying cofactors for HPV in the etiology of cervical cancer have relied primarily on case-control designs because of the long latency between HPV infection and cancer development and the relatively infrequent occurrence of cervical cancer. The longest prospective cohort studies of HPV-infected women are still <15 years old and have not observed virtually any cases of invasive disease as the result of treatment of intraepithelial precursors. The major suspected cofactors based on available data include host susceptibility and behavioral influences (3, 4). The behavioral cofactors implicated by the case-control studies include multiparity, smoking, long-term oral contraceptive (OC) use, chronic infection with other sexually transmitted diseases or inflammation, and possibly nutritional deficiencies (3, 4).

The proper choice of controls for case-control studies of HPV cofactors is particularly challenging. The impact of a factor on the risk of getting infected cannot be ignored when constructing a coherent multistage model of cervical carcinogenesis. However, cervical cancer does not develop in the absence of HPV infection; therefore, only controls exposed to oncogenic types of HPV should be included in an investigation of cofactors for neoplastic progression. Detection of HPV DNA is the reference standard of infection, but many more women are exposed than DNA assays (which measure current infection) indicate (5). The peak prevalence of oncogenic HPVs infecting the cervix occurs among young women initiating sexual intercourse with new partners in their teens and twenties, whereas the median age of cervical cancer diagnosis is two decades later (6). Thus, most previously exposed women with controlled infections will

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test DNA negative. A 40-year-old, HPV DNA-positive control is also not necessarily a good comparison for a case of the same age because it is unusual for women to remain HPV positive but free of significant neoplasia for many years. The ideal control group would be composed of a more thorough sample of women exposed to HPV in the past, at the same age as the comparable cases, but without neoplastic outcome. Unfortunately, no available biomarker permits ideal control selection.

In the absence of a perfect choice, it is worth comparing results using different approaches to measuring HPV infection. HPV virus-like particle serology is a useful tool for the identification of women previously exposed to HPV. Although the assays are not highly sensitive (not all exposed women seroconvert), they are specific for infection with individual HPV types (5). Therefore, restricting analyses to seropositive women represents a reasonable approach to controlling for confounding by HPV exposure in case-control studies of cofactors.

We conducted a large case-control study of invasive cervical cancer in five areas of the United States in the early 1980s, from which sera were stored. Initially, the study was analyzed without taking into account the role of HPV infection, which was unclear at that time. No assays were available and no DNA was collected (7). However, the interview was detailed and is still relevant. Now that reliable serologic assays are available, we tested the stored sera for evidence of exposure to the most common oncogenic types of HPV. We compared the cases with the seropositive controls to clarify the importance of cofactors for cervical cancer among HPV-exposed women.

Materials and Methods

Population. Study subjects were drawn from a case-control study that has been described previously (7). Five cities (Birmingham, Chicago, Denver, Miami, and Philadelphia) reporting to the Comprehensive Cancer Patient Data System were chosen as study sites. Women ages 20 to 74 years diagnosed with incident invasive cervical cancer in 24 hospitals were recruited during April 1982 to January 1984. Community controls were obtained through random digit dialing. Two controls were individually matched to each case by 5-year age group, race, and telephone exchange. Trained interviewers conducted home interviews to collect information on demographics, sexual behavior, smoking, reproductive history, hygiene, contraceptive use, medical history, diet, marital status, and family history of cancer.

With regard to participation, 481 of 658 (73.1%) eligible cases and 801 of 1,114 (71.9%) eligible controls completed interviews. Reasons for nonresponse included refusal (9.7% of cases, 21.9% of controls), subject moved or unable to locate subject (3.8% of cases, 3.4% of controls), death (5 % of cases, 0.5% of controls), illness (2.1% of cases, 1.1% of controls), other problems (1.7% of cases, 1.1% of controls), and failure to obtain physician consent (4.6% of cases). Thirty-five percent of cases were interviewed within 3 months of diagnosis; the remainder was interviewed within 6 months of diagnosis. All participants provided written informed consent.

Cases were classified as squamous cell carcinomas, adenocarcinomas, and adenosquamous carcinomas based on hospital pathology reports. The current study includes as cases only women with squamous cell carcinoma (Fig. 1). Seventy-nine controls that were originally matched to the nonsquamous cases were excluded, leaving 722 eligible controls. Among the interviewed study participants, 235 of 418 (56.2%) women with squamous cell carcinoma and 486 of 722 (67.3%) controls donated blood that was used to test for HPV. Thus, we explored whether there were differences in participation between cases and controls that might lead to bias (8). Specifically, we compared odds ratios (OR) among all the interviewed subjects and among those participating in the blood draw. Similar patterns of risk were seen (data not shown), suggesting that participation bias was minimal.

HPV Serologic Testing. The serologic testing was conducted in two phases in different laboratories. A HPV-16 virus-like particle ELISA was used to test for HPV-16 serum antibodies, as described elsewhere (9). Between-batch and between-day variabilities were controlled by adjusting the absorbance reading of each sample using measurements from three control samples that were run in triplicate in each batch. An absorbance of >0.904 was considered seropositive for HPV-16 (9).

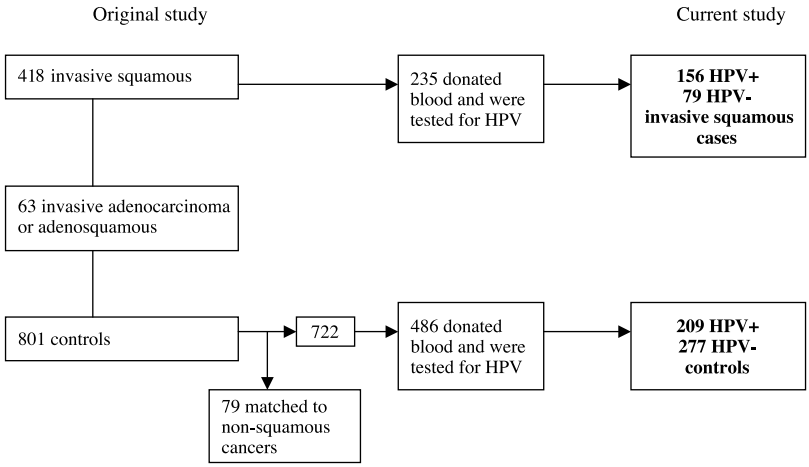

Figure 1. Study subject selection.

Antibodies to HPV types 18, 31, 45, and 52 were tested using a polymer-based virus-like particle ELISA. The assay was done as described previously (10). The cut point for each HPV type-specific virus-like particle ELISA was determined using sera from a panel of HPV DNA-negative young women who reported being virgins and a group with HPV DNA type-specific infection for either 18, 31, 45, or 52. Receiver operating characteristic analyses for all four HPV types had an optimal sensitivity and specificity using an absorbance of >0.16, which was used as the cut point for seropositivity in this study. Each plate tested included a known positive and a known weakly reactive control. In addition, blinded quality control samples, one positive and three negative, were included in each batch. The final absorbance values were not adjusted because minimal between-batch variability was seen.

The use of serology was meant to define a stratum of controls that had definitely been exposed to the main causal agent, namely, oncogenic HPV infection. To assure further that the seropositive controls were accurately characterized, we did subanalyses using more stringent cut points for seropositivity of the five HPV types (HPV-16 absorbance > 1.017; HPV 18, 31, 45, and 52 absorbance > 0.3 considered seropositive). We reached similar conclusions; thus, only the main analyses are shown.

Statistical Methods. Variables available for analysis included demographic variables, screening variables, sexual behavior variables, genital infections, OC variables, other birth control variables, reproductive variables, hygiene variables, and smoking variables. Specific variables are detailed in Results.

All analyses were done using Stata 7.0 (StataCorp, College Station, TX). The first set of analyses was done using all 486 controls that were tested for HPV. We calculated prevalence ORs to determine the risk of being seropositive to one type of HPV given seropositivity to another type. Determinants of seropositivity among controls were tested by standard heterogeneity χ^2 and trend tests.

Based on knowledge that HPV serology is not 100% sensitive, and the results of very large international case series showing that virtually all cases are HPV DNA positive, we considered all 235 cases to be HPV exposed (11). In a confirmatory case-control subanalysis, we looked at risk factors for cervical cancer restricted to the 156 cases that tested seropositive. Relative risks were estimated by ORs obtained from unconditional logistic regression. Tests for trend were obtained by assigning ordinal values to each level of the variable and treating the ordinal variable as a continuous variable in the model.

Results

Overall, 66.4% of cases and 43.0% of controls tested positive for antibodies to at least one of the five HPV types tested (Table 1). Cases were more likely to test positive for each of the five types than were controls. Among controls, seropositivity to one HPV type was strongly associated with seropositivity to other types with ORs ranging from 5.8 to 14.5 (Table 2).

Selected determinants of seropositivity among the 486 controls are presented in Table 3. Increased seropositivity was related to Black race and possibly to Hispanic ethnicity, independent of income. The strongest predictor of seropositivity was higher lifetime numbers of sexual partners; women who had a high lifetime number of partners tended to have mainly nonregular partners. OC use was marginally associated with increased HPV seroprevalence, whereas long duration of condom use was associated with decreased seroprevalence. Increasing parity was also linked to increased seropositivity. HPV seropositivity was not at all associated with any measure of smoking (ever/never, intensity, duration, or recency), with vaginal discharge, or with yeast infections. Few women reported specific sexually transmitted infections.

To assess the influence of demographic and sexual variables independent of HPV exposure, we restricted subsequent analyses to HPV-exposed women (all cases, seropositive controls). These analyses showed no residual effect of sexual behavior, but relationships with several other possibly interrelated factors remained. First, Papanicolaou (Pap) smear screening remained an important predictor, as expected (Table 4).

Black women, although relatively more likely to be seropositive as shown previously in Table 3, were at decreased risk of cervical cancer relative to White, non-Hispanic women once analyses were restricted to HPV-exposed women (Table 4). Women with a history of nonspecific genital infection were at increased risk of cervical cancer relative to women with no history, whereas women who had ever had a yeast infection were at decreased risk compared with women without such a history.

Among HPV-exposed women as assessed in our population by serology, the risk of cervical cancer decreased with increasing years of OC use. Of the other birth control methods assessed, only intrauterine device use was significantly related to cervical cancer risk, with women using an intrauterine device for an increasing number of years at decreased risk. Mutual adjustment of OC and intrauterine device use did not change the risk relationships, with both linked to decreased risk of cancer among HPV-exposed women.

Women with greater numbers of live births were at increased risk of cervical cancer and women with a younger age at first birth were at somewhat increased risk relative to women with an older age at first birth. The increased risk associated with a younger age at first birth was diminished after adjusting for number of live births (data not shown). In turn, the trend of

Table 1. Seropositivity in cases and controls

HPV type	Seropositivity [*n* (%)]	
	Cases (*n* = 235)	Controls (*n* = 486)
16, 18, 31, 45, or 52	156 (66.4)	209 (43.0)
16	91 (38.7)	86 (17.7)
18	86 (36.6)	111 (22.8)
31	93 (39.6)	121 (24.9)
45	54 (23.0)	69 (14.2)
52	43 (18.3)	51 (10.5)

Table 2. Prevalence OR (95% confidence interval) of seropositivity to one HPV type versus seropositivity to another HPV type among controls

Seropositivity	Seropositivity			
	HPV-16	HPV-18	HPV-31	HPV-45
HPV-18	5.9 (3.6-9.8)			
HPV-31	7.3 (4.4-12.1)	10.9 (6.7-17.6)		
HPV-45	5.8 (3.3-10.1)	11.8 (6.7-20.9)	6.7 (3.9-11.4)	
HPV-52	9.7 (5.2-18.1)	11.5 (6.0-22.1)	14.0 (7.0-27.7)	14.5 (7.6-27.7)

NOTE: To compute each OR, the presence/absence of a type in the presence/absence of another was compared in a paired 2 × 2 table.

increasing risk of cervical cancer with greater number of live births was weakened but not eliminated after adjustment for demographics and screening (≥5 versus 0 births: OR, 1.6; 95% confidence interval 0.7-3.7; *P* for trend = 0.18).

Smoking was associated with an increased risk of cervical cancer, with a greater elevation in risk for current than former smokers. There were trends of increasing risk with greater number of cigarettes smoked and greater number of years smoked. In a model containing variables for number of cigarettes and years of smoking among smokers, both trends of increasing risk remained significant and were apparent in both former and current smokers.

To construct a final multivariable model predicting the risk of cervical cancer among HPV-exposed women (Table 5), all variables with significant associations in Table 4 were included. The final model was also adjusted for the matching variables age and race, and multiparity because of the widespread *a priori* interest in this last

Table 3. Major determinants of seropositivity in controls

	n	% Seropositive	Prevalence OR (Univariate)	Prevalence [OR (95% Confidence interval; multivariate)]*
Age (y)				
<35	120	49.2	1.0	1.0
35-44	141	44.0	0.8	0.9 (0.5-1.7)
45-54	101	41.6	0.7	0.9 (0.4-1.9)
≥55	124	37.1	0.6	1.0 (0.5-2.1)
Race				
White, non-Hispanic	321	34.9	1.0	1.0
White, Hispanic	31	38.7	1.2	1.6 (0.7-3.8)
Black	132	63.6	3.3	2.0 (1.2-3.5)
Asian/American Indian	2	50.0	NA	NA
Family income ($/y)				
<5,000	49	61.2	1.0	1.0
5,001-10,000	59	42.4	0.5	0.6 (0.2-1.5)
10,001-20,000	138	49.3	0.6	0.9 (0.4-2.1)
≥20,001	227	34.8	0.3	0.6 (0.3-1.3)
Unknown	13	53.9	NA	NA
Lifetime sexual partners				
1	190	25.3	1.0	1.0
2	79	36.7	1.7	1.5 (0.8-2.7)
3-4	90	51.1	3.1	2.9 (1.6-5.2)
5-9	68	58.8	4.2	2.6 (1.3-5.1)
≥10	57	79.0	11.1	7.7 (3.4-17.0)
Years of OC use				
0	228	35.5	1.0	1.0
<5	150	46.0	1.5	1.4 (0.9-2.6)
5-10	60	50.0	1.8	1.9 (0.9-4.1)
>10	40	65.0	3.4	2.4 (1.0-5.7)
Years of condom use				
0	314	45.2	1.0	1.0
≤1	51	39.2	0.8	0.6 (0.3-1.3)
>1-5	54	50.0	1.2	1.1 (0.6-2.1)
>5	59	28.8	0.5	0.5 (0.2-1.0)
No. live births				
0	79	39.2	1.0	1.0
1	76	42.1	1.1	0.9 (0.4-1.8)
2	123	45.5	1.3	1.6 (0.8-3.3)
3	96	32.3	0.7	1.0 (0.5-2.0)
4	47	51.1	1.6	2.2 (0.9-5.4)
≥5	65	53.9	1.8	2.0 (0.9-4.5)

*Adjusted for all variables in table.

Table 4. Estimated relative risk of cervical cancer among HPV-positive women

	Cases	Controls	OR	95% Confidence interval
Demographics				
Age (y)				
<35	51	59	1.0	
35-44	66	62	1.2	0.7-2.1
45-54	60	42	1.7	1.0-2.9
≥55	58	46	1.5	0.9-2.5
Race				
White, non-Hispanic	141	112	1.0	
White, Hispanic	22	12	1.5	0.7-3.1
Black	65	84	0.6	0.4-0.9
Asian/American Indian	6	1	4.8	0.6-40.2
Education (y)				
<9	43	25	1.0	
9-11	69	31	1.3	0.7-2.5
12	66	66	0.6	0.3-1.1
≥13	57	87	0.4	0.2-0.7
Income ($/y)				
<5,000	61	30	1.0	
5,001-10,000	44	25	0.9	0.5-1.7
10,001-20,000	47	68	0.3	0.2-0.6
≥20,001	76	79	0.5	0.3-0.8
Unknown	7	7	0.5	0.2-1.5
Screening				
Years since last Pap				
≤2	117	160	1.0	
3-4	25	21	1.6	0.9-3.0
5-9	28	6	6.4	2.6-15.9
≥10	28	6	6.4	2.6-15.9
Never	34	12	3.9	1.9-7.8
Unknown	3	4	1.0	0.2-4.7
Abnormal Pap				
Never	181	180	1.0	
Ever	50	25	2.0	1.2-3.4
Sex-related variables				
Lifetime sexual partners				
1	57	48	1.0	
2	41	29	1.2	0.7-2.2
3-4	52	46	1.0	0.6-1.7
5-9	43	40	0.9	0.5-1.6
≥10	40	45	0.8	0.4-1.3
Years since first intercourse				
≤14	38	42	1.0	
15-24	66	73	1.0	0.6-1.7
25-34	58	34	1.9	1.0-3.5
≥35	71	59	1.3	0.8-2.3
History of nonspecific genital infection				
Never	199	195	1.0	
Ever	36	14	2.5	1.3-4.8
Yeast infection				
Never	174	118	1.0	
Ever	61	91	0.5	0.3-0.7
Contraceptives				
Years of OC use				
0	123	81	1.0	
<5	59	69	0.6	0.4-0.9
5-10	33	30	0.7	0.4-1.3
>10	20	26	0.5	0.3-1.0
Years of intrauterine device use				
0	204	158	1.0	
<5	23	30	0.6	0.3-1.1
≥5	8	18	0.3	0.2-0.8
Reproductive factors				
No. live births				
0	25	31	1.0	
1	34	32	1.3	0.7-2.7

(Continued on the following page)

Table 4. Estimated relative risk of cervical cancer among HPV-positive women (Cont'd)

	Cases	Controls	OR	95% Confidence interval
2	45	56	1.0	0.5-1.9
3	39	31	1.6	0.8-3.2
4	36	24	1.9	0.9-3.9
≥5	56	35	2.0	1.0-3.9
Age at first birth (y)				
≥22	69	72	1.0	
20-21	41	34	1.3	0.7-2.2
18-19	49	36	1.4	0.8-2.4
<18	52	37	1.5	0.9-2.5
Smoking				
Smoking currency				
Never	93	110	1.0	
Former	39	33	1.4	0.8-2.4
Current	103	66	1.9	1.2-2.8
Cigarettes per day (among smokers)				
<10	14	23	1.0	
10-19	32	26	2.0	0.9-4.7
20-29	56	31	3.0	1.3-6.6
>30	40	19	3.5	1.5-8.2
Years of smoking (among smokers)				
<10	23	30	1.0	
10-19	32	24	1.7	0.8-3.7
20-29	38	20	2.5	1.2-5.3
30-39	32	16	2.6	1.2-5.9
≥40	17	9	2.5	0.9-6.5
Years since stopped smoking (former smokers)				
1-5	16	11	1.0	
6-14	10	11	0.6	0.2-2.0
≥15	13	11	0.8	0.3-2.5

variable as a HPV cofactor. The final, significant predictors of cervical cancer risk included education, income, Pap smear screening history, nonspecific genital infections, yeast infections, and duration and dose of smoking. OC use, number of live births, and years of intrauterine device use were not independent, significant predictors. Excluding seronegative cases did not alter the results.

Discussion

HPV infection is such a powerful risk factor for cervical cancer that adjustment for its confounding effects is critical in the epidemiologic assessment of etiologic cofactors. There is no perfect approach available for such adjustment. Possible cofactors must be evaluated using a variety of methodologic approaches that vary mainly in the choice of controls.

The accumulated data regarding possible risk factors for cervical cancer among HPV-infected women are derived mainly from case-control studies based on DNA testing to define controls (12, 13). This study was conducted prior to the availability of any HPV test method (7). We conducted the present analyses to see whether an alternative approach, the use of serologic testing for HPV in a case-control study with an extensive questionnaire, could help clarify the cofactors for development of cervical cancer among HPV-exposed women in the United States.

The major case-control studies controlling for HPV infection using HPV DNA testing have implicated smoking, OC use, multiparity, and coinfections or inflammation as risk factors for cervical cancer. Among women exposed to oncogenic HPV infection as indicated by serology, we too observed a convincing elevation of risk associated with increasing intensity and duration of smoking. The data suggested that women using OCs and those with high parity might be at increased risk of HPV infection but did not confirm a definite role for these factors in the development of cancer once women had been infected.

Virus-like particle serology has been proven in this investigation to be a believable marker of past exposure to HPV, permitting the reliable delineation of a HPV-positive stratum that could be properly compared with cases. We are not suggesting that seronegative controls were necessarily unexposed to oncogenic HPV, even to the four types for which we assayed. At present, there is no accurate measurement of lifetime HPV exposure. We used serology to define a reasonably large HPV-exposed stratum and controlled for the possibly powerful confounding effects of HPV exposure by exclusion of the seronegative controls. The performance of the assay for this limited purpose seemed adequate. The assay was reproducible and restriction to a more stringent cut point for seropositivity led to the same conclusions. The seroprevalence among cases was reassuringly high. Although few studies have assessed seroprevalence of multiple HPV types in the United States, our findings that 17.7% of controls and 38.7% of cases were seropositive for HPV-16 are similar to those of a previous population-based survey in the United States (ref. 14; 17.9% of women seropositive for HPV-16) and a U.S.-based case-control study (ref. 15; 10.5% of controls, 23.8% of cases seropositive for HPV-16). We found that seropositivity to one HPV type was related to seropositivity

to other types. Seropositivity to multiple types probably reflects that women are often concurrently or sequentially infected with multiple HPV types rather than cross-reactivity of the assays (5, 16, 17).

Moreover, the use of serology permitted an interesting exploration of the epidemiology of HPV infection in the United States in the early 1980s. We saw, as expected from others' work, a strong association of seropositivity with measures of sexual behavior, particularly a greater lifetime number of sexual partners (14, 17-19). Long-term condom use was somewhat protective. We also noted strong epidemiologic associations of HPV seropositivity with African American race and low socioeconomic status, which is consistent with historical associations of these sociodemographic variables with cervical cancer risk. This study and others observed only weak associations between age and seropositivity, suggesting perhaps that seropositivity resulting from early exposures to HPV at the start of sexual activity wanes slowly over time, although cohort effects are also a possibility (5, 16-18).

Among HPV-exposed women (assuming based on a huge literature that virtually all cases were infected), the number of lifetime sexual partners was no longer associated with cervical cancer, clearly illustrating that the increased risk of cervical cancer associated with sexual behavior largely reflects the probability of exposure to HPV, the central causal agent. However, the sexual behavioral variable that we assessed is too crude to rule out a residual association of sexual behavior with risk of neoplasia. A limited role for sexual behavior in women already infected with HPV might still exist if infection with other sexually transmitted infections and/or chronic inflammation is important. Too few women reported other sexually transmitted infections to assess this possibility in our data. Schmauz et al. (20) observed a trend of increased risk for cervical cancer with increasing number of infections. More recent studies found associations between *Chlamydia trachomatis* and/or herpes simplex virus-2 and cervical cancer risk among HPV DNA-positive women, although the evidence is not uniformly supportive (21, 22). It has been hypothesized that chronic infection with various sexually transmitted infections may act via inflammation of the cervix leading to genotoxic damage via reactive oxidative metabolites (23). Our observation of an increased risk of cervical cancer among women reporting a history of nonspecific genital infections supports the idea that chronic infection with various sexually transmitted infections may contribute to cervical cancer risk. It is unclear, even after extensive multivariate analyses taking into account the other variables in the interview, why we observed a decreased risk of cervical cancer among women with previous yeast infections, which were not associated with HPV seropositivity.

Black women and, to a less extent, Hispanic women were at increased risk of exposure to HPV. However, Black women were at decreased risk of cervical cancer once analyses were restricted to those exposed. This might be chance, but it certainly suggests that the major

Table 5. Multivariate model of relative risk of cervical cancer among HPV-positive women

	Cases	Controls	Adjusted OR*	95% Confidence interval
Age (y)				
≤44	117	121	1.0	
≥45	118	88	0.8	0.5-1.3
Race				
White, non-Hispanic	141	112	1.0	
Black	65	84	0.4	0.2-0.7
Other	28	13	1.8	0.8-4.0
Education (y)				
<12	112	56	1.0	
≥12	123	153	0.5	0.3-0.8
Income ($/y)				
≤10,000	105	55	1.0	
>10,000/unknown	130	154	0.6	0.3-1.0
Pap smear screening				
≤4 y ago/never abnormal	96	157	1.0	
≤4 y ago/abnormal	45	24	4.0	2.1-7.5
>4 y ago/never abnormal	51	11	5.8	2.7-12.3
>4 y ago/abnormal	5	1	8.8	1.0-81.7
Never	34	12	3.4	1.5-7.6
Unknown	3	4	1.3	0.3-6.6
History of nonspecific genital infection				
Never	199	195	1.0	
Ever	36	14	4.6	2.2-9.9
Yeast infection				
Never	174	118	1.0	
Ever	61	91	0.5	0.3-0.8
Smoking				
Never	93	110	1.0	
<20 cigarettes/d/<20 y	22	27	1.2	0.6-2.4
<20 cigarettes/d/≥20 y	24	22	1.2	0.5-2.7
≥20 cigarettes/d/<20 y	33	27	1.6	0.8-3.3
≥20 cigarettes/d/≥20 y	63	23	2.8	1.5-5.3

*Adjusted for all other variables in table.

determinants of historically elevated rates among Black women are behavioral rather than genetic. Low socioeconomic status was associated with both HPV exposure and cancer risk among the exposed, as a proxy for unknown other variables.

Findings of an increased risk of cervical cancer among long-term OC users have prompted calls for increased efforts to screen long-term OC users and even consideration of recommendation of alternative birth control methods (24, 25). In the original analysis of this data set, with no direct measure of HPV, OC use was not associated with cervical cancer risk in univariate analyses, after adjustment for other variables, particularly Pap smear screening, however, women who used OCs for >5 years were at 2-fold increased risk of cervical cancer relative to nonusers (P for trend = 0.003; ref. 7). HPV is responsive *in vitro* to steroid hormones; thus, an effect of steroid contraceptives on HPV natural history and risk of neoplasia is plausible (26). However, in the present analysis, among HPV-exposed women as defined by serology, long-term OC use was associated with a decreased risk of cervical cancer, an association that was no longer apparent after adjustment for demographic and Pap smear screening variables. In attempting to understand the differences in the past and present analyses, we noted that women who used OCs were more likely to be seropositive than nonusers, a result similar to those of several previous investigations (5, 14, 17). Unlike previous studies (14), the association between OCs and seropositivity persisted after adjustment for lifetime number of sexual partners in this study. Conceivably, OC use might increase cervical cancer risk via a causal intermediate that also leads to increased seroprevalence (e.g., viral persistence). If true, restricting our analysis of the risk of OCs to seropositive women would bias our estimates of risk toward the null due to ''unknowing, partial statistical adjustment for a causal intermediate.'' Alternatively, we recognize that the serologic assay is not a perfectly sensitive measurement of lifetime HPV exposure. If OC use increases seropositivity (with no causal implications regarding cancer), by conducting our cofactor analysis only among seropositive controls, we would bias our risk estimate toward the null. Without more information regarding the associations of OCs, seropositivity, and cervical cancer risk, we cannot determine why our findings differ from those based on HPV DNA testing. In ongoing prospective analyses, we are testing HPV DNA, serology, OC use, and incident neoplasia to disentangle these relationships. As another concern related to the evaluation of OCs in the current study, we lacked the statistical power to assess adequately the major public health concern, which is the effect of prolonged OC use in the absence of screening.

In studies restricted to HPV DNA-positive women, high parity has been consistently associated with an increased risk of cervical cancer in populations with high parity (13, 27), but generally no association is seen in populations with low parity (28, 29). In this U.S. population, we found some evidence of a relationship of multiparity with HPV exposure and with cervical cancer risk once exposure had occurred. However, the effect on cancer risk among HPV-exposed women was diminished toward the null, to statistical nonsignificance, after adjustment for screening and demographic variables. As with OCs, this might indicate that multiparity increases cervical cancer risk via a mechanism that also increases seroconversion. Again, a possible role of hormonal influences on persistence could be imagined. These conjectures must be corroborated with more direct evidence.

Among HPV-exposed women, we observed, as have others, an increased risk of cervical cancer among women who smoked, particularly those who currently smoke (28, 30). Overall, we conclude that the etiologic role of smoking as a HPV cofactor is robust regardless of study design and control selection. It is unclear whether the risk of cervical cancer posed by smoking is immunologic (reducing HPV clearance) or genotoxic (more directly promoting neoplastic progression).

The use of serologically defined controls corroborated many of the conclusions of studies based on HPV DNA testing. Given the public health importance of the issue, there may still be a particular need to evaluate further using all available methods how OC use affects HPV natural history, host response, and risk of cervical neoplasia.

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